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CROP PRODUCTION

Evolution of nutrient availability in maturation phase of composting using proportions of different residues inoculated with *Beijerinckia indica*

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ABSTRACT. Organic matter has low N content; however, organic matter may be enriched by inoculation with selected diazotrophic bacteria. The aim of the paper was to evaluate the effects of biofertilizer produced by mixing different types and proportions of organic matter inoculated with the diazotrophic bacteria *Beijerinckia indica.* The experiment consisted of plastic trays (6 L) containing 5 kg of three different types of organic matter (filter mud cake, earthworm compost, and crop residue) applied in the following proportions (v:v:v): 1-(5:0:0), 2-(4:1:0), 3-(4:0:1), 4-(3:2:0), 5-(3:1:1), 6-(3:0:2), 7-(2:3:0), 8-(2:2:1), 9-(2:1:2), 10-(2:0:3), 11-(1:4:0), 12-(1:3:1), 13-(1:2:2), 14-(1:1:3), 15-(1:0:4), 16-(0:5:0), 17-(0:4:1), 18-(0:1:4), 19-(0:3:2), 20-(0:2:3), and 21-(0:0:5). Samples were collected following inoculation with *B. indica* at 0, 10, 20, 30, and 40 days of the maturation phase. The chemical analyses were: pH (H_2O), total C and N, available P and K, and exchangeable Na^+, Ca^{+2}, Mg^{+2}. The treatments with the best results showed significant effects following the maturation phase. The exceptions were C and N, which presented only individual effects. In general, the organic matter proportions (5:0:0), (3:2:0), and (2:3:0) resulted in a significant increase in the availability of nutrients. Biofertilizers with optimal organic matter proportions may be used to produce organic substrates that are more effective and have the potential to be applied as alternatives to soluble NPK fertilizers.

Keywords: *Acidithiobacillus*; diazotrophic bacteria; earthworm compost; plant residue; sugarcane filter mud cake.

Evolução da disponibilidade de nutrientes na fase de maturação da compostagem usando diferentes proporções de resíduos inoculados com *Beijerinckia indica*

RESUMO. A matéria orgânica de uma maneira geral apresenta baixa concentração de N; entretanto, material orgânico pode ser enriquecido por inoculação com bactérias diazotróficas selecionadas. O objetivo principal da pesquisa foi de avaliar os efeitos na produção de biofertilizante produzido com a mistura de vários materiais aplicados em diferentes proporções inoculados com a bactéria diazotrófica *Beijerinckia indica*, selecionada em trabalho prévio. O experimento utilizou vasos plásticos (6 L) contendo 5 kg da mistura de três diferentes materiais orgânicos (torta de filtro de usina, húmus de minhoca e resíduo de cultura) aplicados nas seguintes proporções (v:v:v): 1-(5:0:0), 2-(4:1:0), 3-(4:0:1), 4-(3:2:0), 5-(3:1:1), 6-(3:0:2), 7-(2:3:0), 8-(2:2:1), 9-(2:1:2), 10-(2:0:3), 11-(1:4:0), 12-(1:3:1), 13-(1:2:2), 14-(1:1:3), 15-(1:0:4), 16-(0:5:0), 17-(0:4:1), 18-(0:1:4) 19-(0:3:2), 20-(0:2:3) e 21-(0:0:5). Amostras foram coletadas após inoculação com *B. indica* durante o período de 0, 10, 20, 30, e 40 dias na fase de maturação. As análises químicas realizadas foram: pH (H_2O), C e N total, P e K disponível, e Na^+, Ca^{+2}, Mg^{+2} trocáveis. Os tratamentos com os melhores resultados mostraram interação com efeitos significativos nas diferentes fases de maturação, com exceção do C e N total que só apresentaram efeitos individuais. De uma maneira geral as melhores proporções de materiais orgânicos foram (5:0:0), (3:2:0) e (2:3:0) que mostraram aumento significativo na disponibilidade de nutrientes. Biofertilizantes com melhores proporções de material orgânico podem ser utilizados para produzir substratos orgânicos mais efetivos e com potencial para uso alternativo a fertilizantes comerciais.

Palavras-chave: *Acidithiobacillus*; bactéria diazotrófica; húmus de minhoca; resíduo de plantas; torta de filtro de cana de açúcar.

Introduction

Biofertilizer produced with organic matter (earthworm compost) has been shown to increase the availability of nutrients in various crops, especially phosphorus, potassium, calcium, magnesium, and sulfur (Lima, Stamford, Santos, Lira Junior, & Dias, 2007; Stamford, Lima, Santos, & Dias, 2006; Stamford, Izquierdo, Fernández, & Moreno, 2008; Stamford et al., 2011). Nitrogen is also present in organic matter, although not in quantities that promote adequate nutrition and plant yield similar to an application of soluble fertilizer (Lima et al., 2010). However, organic matter inoculated with effective free-living diazotrophic bacteria can promote the production of N to acceptable levels (Lima et al., 2010).

The production of soluble NPK fertilizers first requires that they are chemically processed, resulting in high concentrations of readily available nutrients. Additionally, the production of soluble fertilizers often requires the addition of high temperatures or the use of strong acids (sulfuric acid, phosphoric acid or nitric acid), and these processes require high power consumption (Van Straaten, 2002).

Because of these requirements, organic matter seems to be a viable alternative for small, medium and large farms due to its low cost. Natural organic fertilizers can be produced without the use of corrosive products or high-tech equipment, using simple field techniques (Stamford et al., 2008).

The incorporation of organic matter generally improves the physical conditions of the soil and contributes to increased biological activity that provides nutrients to plants (Sousa et al., 2015). The primary materials used to produce organic biofertilizers include earthworm compost (Oliveira et al., 2015), crop compounds (Sousa et al., 2015), sugarcane filter mud cake (Oliveira et al., 2015), sugarcane bagasse, straw carbonized rice (Saideles, Winckler, Schirmer, & Sperandio, 2009), and others. Organic waste that has minimal environmental impact is preferred for biofertilizer production (Figueiredo & Tanamati, 2010). It is important to note that these materials must be evaluated to determine the appropriate proportion of each organic material for the production of biofertilizer to ensure nutrient availability and their usefulness in agriculture.

The aim of this paper was to evaluate the best proportions of different types of organic matter inoculated with effective free-living diazotrophic bacteria that would result in increased N and nutrient availability for the production of organic biofertilizer. The ability of the biofertilizers to be used as alternative fertilizer sources that could replace soluble fertilizers was also examined.

Material and methods

The experiment was performed at the Laboratory of Environmental Biotechnology of the Department of Agronomy, Federal Rural University of Pernambuco, Brazil. Using plastic trays (6 dm^3), 5 dm^3 of organic matter (sugarcane mud cake: earthworm compost: crop residue) was mixed in the following proportions (v:v:v): 1-(5:0:0), 2-(4:1:0), 3-(4:0:1), 4-(3:2:0), 5-(3:1:1), 6-(3:0:2), 7-(2:3:0), 8-(2:2:1), 9-(2:1:2), 10-(2:0:3), 11-(1:4:0), 12-(1:3:1), 13-(1:2:2), 14-(1:1:3), 15-(1:0:4), 16-(0:5:0), 17-(0:4:1), 18-(0:1:4), 19-(0:3:2), 20-(0:2:3), and 21-(0:0:5). The experiment was conducted as a complete randomized design using the proportions of the three types of organic matter as treatments and replicated four times.

The sugarcane mud cake was purchased from the "Petribu sugarcane industry", and the chemical analyzes showed: pH (H_2O) = 6.4, total C = 112 (g kg^{-1}), total N = 8.0 (g kg^{-1}), C:N = 14.0, available P = 150 (mg kg^{-1}), and available K = 119 (mg kg^{-1}); The earthworm compost was commercially available in the regional market (Febras Company): pH (H_2O)= 7.9, total C = 134 (g kg^{-1}), total N = 6.9 (g kg^{-1}), C:N = 19.4, available P = 120 (mg kg^{-1}), and available K = 425 (mg kg^{-1}); and the residue compost obtained at the District of Recife City, and the chemical analyzes showed: pH (H_2O) = 6.2, total C = 180 (g kg^{-1}), total N = 4.7 (g kg^{-1}), C:N = 38.2, available P = 80 (mg kg^{-1}), and available K = 159 (mg kg^{-1}).

After the respective treatments were mixed in the plastic trays, they were inoculated with the effective free-living bacteria *Beijerinckia indica* (strain NFB 10001), a strain selected from a previous study (Lima et al., 2010) and further characterized by the Macrogen Incorporation (Geumcheon-gu Seoul, Korea).

The pre-inoculum was initially prepared in a 250 mL Erlenmeyer flask, containing 100 mL of LG medium and incubated for two weeks under orbital agitation (500 gpm). After this period, the material was transferred to a 2,000 mL Erlenmeyer flask with 1,000 mL of LG medium to produce the liquid inoculum and incubated for 7 days with orbital agitation (500 gpm). Each tray received 200 mL of the liquid inoculum, which was homogenized with a sterile spatula. Treatments were incubated at room temperature (28 ± 2°C). The humidity control was set at maximum water retention capacity for each tray, and the daily addition of tap water passed through an activated carbon filter.

During the experiment, samples from each treatment were collected at various times during the maturation phase (0, 10, 20, 30, and 40 days) and chemical analyses of pH (H_2O), total C and N, available P and K, and exchangeable Ca, Mg and Na were all conducted following the Embrapa (2009) methodology.

The data for treatments deemed the most economically viable were statistically analyzed using SAS Institute (2011) software. Logarithmic and square root transformations of the data were not necessary to ensure the normality and homogeneity of the variance. For comparison between treatments, Tukey tests were carried ($p < 0.05$) and all statistical tests were evaluated at the 95% confidence level and values are given as mean ± standard error. Correlation analyses were also not carried out to examine the relationships.

Results and discussion

pH values

The pH values of the selected treatments with different proportions of the 3 types of organic matter (mud cake, earthworm compost and crop residue) at various incubation times are shown in Table 1. A low pH value at time zero (T_0) was observed in treatment 7 (1:1:3), and the other treatments with different proportions were not significantly different when compared at the beginning of the experiment (T_0). The low variation may be due to the amount of sugarcane mud cake and earthworm compost because these types of organic matter have higher pH values (6.7 and 7.9, respectively).

Table 1. The pH values of selected treatments with different proportions of the 3 types of organic matter (mud cake: earthworm compost: crop residue), inoculated with the diazotrophic bacteria *Beijerinckia indica* (strain NFB 10001), after incubation at various times (0, 10, 20, 30, and 40 days) at room temperature (28 ± 2°C).

Treatments	Incubation time (days)				
Proportions	0	10	20	30	40
	pH (H_2O)				
1 (5-0-0)	6.97a±0.12	7.06b± 0.09	7.06b± 0.01	7.16a± 0.06	7.15a± 0.00
2 (2-3-0)	6.61a± 0.01	7.25a± 0.12	7.29a± 0.06	7.31a± 0.09	7.25a± 0.12
3 (3-2-0)	6.90a± 0.34	7.28a± 0.11	7.29a± 0.09	7.34a± 0.02	7.34a± 0.02
4 (3-1-1)	6.81a± 0.02	7.06a± 0.15	7.19a± 0.17	7.25a± 0.07	7.17a± 0.05
5 (2-0-3)	6.68a± 0.15	7.38a± 0.06	7.06b± 0.20	7.14a± 0.12	7.30a± 0.17
6 (2-2-1)	6.73a± 0.04	6.98b± 0.40	7.19a± 0.01	7.27a± 0.09	7.21a± 0.07
7 (1-1-3)	6.41b± 0.24	6.82b± 0.06	6.82b± 0.01	6.76b± 0.02	6.81b± 0.01
8 (1-2-2)	6.63a± 0.00	6.87b± 0.04	7.00b± 0.06	7.02b± 0.09	7.08a± 0.4
9 (0-4-1)	6.75a± 0.02	6.92b± 0.01	6.98b± 0.03	6.80b± 0.22	6.83b± 0.02

Means with the same letter in the same column are not different according to Tukey's test ($p < 0.05$).

A slight increase in pH was observed with increased incubation time, especially from time zero to 30 days of incubation. In general, similar results were observed for the incubation times from 10 to 40 days, with an increase in pH when incubated for 30 days. Higher pH values were observed with treatments (2:3:0) and (3:2:0) at 30 days of incubation; these treatments had a high amount of earthworm compost (pH 7.9).

However, despite the different proportions of the 3 types of organic matter used, these pH values do not pose a problem for the production of organic biofertilizers because the pH values were between 6 and 7. Plant yield and development were not affected when phosphate and potassic rock biofertilizers that have low pH values (pH 3.0-3.5) were mixed with earthworm compost (pH 7.9). Santana et al. (2014) also evaluated the effects of rock biofertilizers mixed with earthworm compost on green pepper yield (*Capsicum annuum*) and observed increases in soil pH and soil nutrient availability after plant harvest.

The effect of mixing organic matter with a high pH, such as earthworm compost (pH 7.9), with rock biofertilizers, which results in a higher soil pH, is very important. Inoculation of rock biofertilizers with the oxidative bacteria *Acidithiobacillus* reduced the pH (3.0-3.5), and the addition of organic matter with a high pH neutralized the acidity, resulting in satisfactory plant growth and yield (Stamford, Stamford, Stamford, Barros Neto, & Takaki, 2007; 2009; Santana et al., 2014; Oliveira et al., 2015).

Exchangeable calcium

The analysis of exchangeable calcium in the treatments with different proportions of the 3 types of organic matter showed significant differences ($p < 0.05$) among the treatments (Table 2).

Table 2. Soluble calcium in the biofertilizers composed of different proportions of the 3 types of organic matter (sugarcane mud cake: earthworm compost: crop residue), inoculated with diazotrophic bacteria *Beijerinckia indica* (strain NFB 10001), after incubation for various maturation phases (0, 10, 20, 30, and 40 days) at room temperature (28 ± 2°C).

Treatments	Incubation time (days)				
(Proportion)	0	10	20	30	40
	Exchangeable calcium (mg dm^{-3})				
1 (5-0-0)	686a± 38	576a± 14	636a± 32	880a±39	570a± 11
2 (2-3-0)	310d±33	457a± 84	387c± 61	452c± 22	398c± 42
3 (3-2-0)	523b±66	378b± 54	423c± 24	480c± 20	448b± 42
4 (3-1-1)	501b±80	534a± 20	579a± 46	715b± 80	544a± 50
5 (2-0-3)	382c±19	333b± 3	486b± 35	369d± 55	503a± 24
6 (2-2-1)	416c±84	493a± 31	498b± 6	820a± 5	457b± 86
7 (1-1-3)	407c±57	456a± 71	542b± 15	475c± 9	424b± 53
8 (1-2-2)	397c±74	409b± 23	336c± 42	489c± 26	276d± 28
9 (0-4-1)	223d±38	324b± 27	315c± 13	371d± 49	304d± 5

Means with the same letter in the same column are not different according to Tukey's test ($p < 0.05$).

The addition of organic matter to the different treatments resulted in increased calcium in those biofertilizers with a high proportion of sugarcane mud cake (5:0:0), probably due to the calcium concentration in this natural substrate. The best results were observed in treatments (5:0:0) and (2:2:1) followed by treatment (3:1:1) when the material was in maturated phase for 30 days.

The results reported by Santana et al. (2014) showed that applying higher rates of mixed biofertilizer (phosphate and potassic rocks mixed with earthworm compost) on green pepper (*Capsicum annuum*) increased exchangeable calcium in the soil compared with a soluble fertilizer application. These results differ from those reported by Stamford et al. (2011), who showed that different fertilization treatments did not differ from one another (mixed biofertilizer, soluble fertilizer, and rock biofertilizer) and who found exchangeable calcium in the soil after two subsequent grapevine harvests.

Rossetto, Dias, and Vitti (2008), in describing the effects of sugarcane mud cake on crop yield, agreed that sugarcane mud cake increases crop yield with the addition of organic matter, phosphorus and calcium. Stamford et al. (2006) reported a significant increase in exchangeable calcium in the soil when applications of phosphate and potassic rock biofertilizer were utilized in sugarcane grown in a tableland Ultisol of northeastern Brazil. Similar results were reported by Stamford et al. (2011) when applying mixed biofertilizer and soluble fertilizer in grape (*Vitis vinifera*) grown in the semiarid region of the San Francisco Valley, Brazil.

Exchangeable magnesium

The treatments with different proportions of the 3 types of organic matter showed significant differences in exchangeable Mg^{+2} in the substrates (Table 3).

Table 3. Soluble magnesium in the biofertilizers with different proportions of the 3 types of organic matter (sugarcane mud cake: earthworm compost: crop residue), inoculated with diazotrophic bacteria *Beijerinckia indica* (strain NFB 10001), after incubation for the maturation phases with various times (0, 10, 20, 30, and 40 days) at room temperature (28 ± 2°C).

Treatments	Incubation time (days)				
(Proportions)	0	10	20	30	40
	Exchangeable magnesium (mg dm^{-3})				
1 (5-0-0)	93a±4	112a ± 9	130a ± 2	187a ± 4	132a ± 1
2 (2-3-0)	66b±9	66c ± 6	59c ± 2	84d ± 7	60d ± 3
3 (3-2-0)	67b±9	57d ± 7	110b ± 5	185a ± 9	115b ± 2
4 (3-1-1)	78b±5	109a ± 2	136a ± 6	167b ± 2	89c ± 9
5 (2-0-3)	56c±4	66c ± 6	60c ± 5	101b ± 9	112b± 5
6 (2-2-1)	62b±7	106a ± 9	107b ± 5	107b ± 7	116b± 9
7 (1-1-3)	47c±8	47d ± 3	98b ± 2	77d ± 1	100c ± 4
8 (1-2-2)	62b±7	57d ± 1	99b ± 9	62e ± 3	39e ± 3
9 (0-4-1)	68b±9	63c ± 1	101b ± 5	106b ± 5	108b ± 7

Means with the same letter in the same columns are not different according to Tukey's test ($p < 0.05$).

The (5:0:0) treatment had higher levels of exchangeable magnesium in the substrates incubated from time zero to 30 days in maturation phase and showed values that were very different compared with the other mixed proportions. The effects of the different proportions on exchangeable magnesium during 10 to 30 days of maturation phase were evident in the proportions (5:0:0), (3:2:0), and (3:1:1). It is important to note that these treatments used sugarcane mud cake in higher proportions. A reduction in exchangeable magnesium was observed during the 40-day of the incubation phase. Santana et al. (2014) did not report significant differences in exchangeable magnesium in the soil when different fertilization treatments were applied.

Other studies have reported an increase in exchangeable magnesium in soil when organic fertilizers are applied (Moura, Stamford, Duenhas, Santos, & Nunes, 2007; Stamford et al., 2006; Stamford et al., 2009; Stamford et al., 2014). The available magnesium may be released from minerals as biotite, which is used in the production of mixed fertilizers with PK rocks and organic matter, especially when biotite and sulfur are inoculated with the oxidative bacteria *Acidithiobacillus*, which produces sulfuric acid and releases magnesium due to acidity.

Available phosphorus

The available phosphorus in the different proportions of the 3 types of organic matter was significantly affected by the various incubation periods of the maturation phase (Table 4).

Table 4. Available phosphorus in the biofertilizers with different proportions of the 3 types of organic matter (sugarcane mud cake: earthworm compost: crop residue), inoculated with the diazotrophic bacteria *Beijerinckia indica* (strain NFB 10001), after incubation for various times in the maturation phase (0, 10, 20, 30, and 40 days) at room temperature (28 ± 2°C).

Treatments	Incubation time (days)				
(Proportions)	0	10	20	30	40
	Available phosphorus (mg dm^{-3})				
1 (5-0-0)	1553d±18	1578c ±20	2264b±90	2867a±38	2337a±33
2 (2-3-0)	1521d±10	1246c±16	1564d±80	2839a±95	1987b±93
3 (3-2-0)	2232a±35	2371a±90	2749a±50	3035a±40	2457a±93
4 (3-1-1)	2253a±46	2300a±31	1907c±40	1976b±63	1946b±66
5 (2-0-3)	1942b±85	1539c±16	2308b±90	1840b±53	1657c±75
6 (2-2-1)	1269d±58	1067c±95	2740a±96	2296b±56	2041a±63
7 (1-1-3)	698e±83	1689b±27	1575d±64	1723c±28	1901b±57
8 (1-2-2)	1795c±31	1214c±72	1214e±40	2278b±23	1308d±43
9 (0-4-1)	1143d±90	1457c±93	2274b±78	1996b±47	1951b±48

Means with the same letter in in the same column are not different according to Tukey's test ($p < 0.05$).

The available P significantly differed in the (3:2:0) and (3:1:1) treatments at time zero (T_0). After 10 and 20 days of incubation in the maturation phase, treatment effects were observed for proportions (3:2:0) and (3:1:1). The highest available P was obtained in the maturation phase correspondent to 30 days in proportions (3:2:0) and (2:3:0). At 40 days of incubation, similar to the tendency observed with the other nutrients, we observed a decrease in the P content.

The availability of phosphorus also depends on the ability of diazotrophic bacteria to fix N, as well as the activity of microorganisms that degrade organic materials, which contributes to an increase in the availability of phosphorus and other nutrients. Furthermore, the addition of organic material to the substrate increases microbial activity by promoting the mineralization of the material, thus allowing an increase in the availability of nutrients in these systems, soil, or organic substrates.

Stamford et al. (2014) evaluated mixed biofertilizers that were produced with PK rocks and organic matter with high N content due to inoculation with diazotrophic bacteria and found an increase in available P in the soil. Similar results were reported by Silva, Stamford, Amorim, Almeida Junior, and Silva (2011), who evaluated melon growth and phosphorus availability in the soil by applying phosphate and potassium biofertilizers after three subsequent cycles.

Available potassium

Available potassium was significantly affected by the different proportions of the 3 types of organic matter inoculated with diazotrophic bacteria during various incubation times of the maturation phase (Table 5).

Table 5. Available potassium in the biofertilizers with different proportions of the 3 types of organic matter (sugarcane mud cake: earthworm compost: crop residue), inoculated with the diazotrophic bacteria *Beijerinckia indica* (strain NFB 10001), after incubation for various times of the maturation phase (0, 10, 20, 30, and 40 days) at room temperature (28 ± 2°C).

Treatment	Incubation time (days)				
(Proportions)	0	10	20	30	40
	Available phosphorus (mg dm^{-3})				
1 (5-0-0)	109e±15	232c±62	201d±44	215d±38	130d±19
2 (2-3-0)	710a±12	639a±63	602b±114	404b±19	380c±84
3 (3-2-0)	519b±53	489b±31	497b±46	303c±73	339c±114
4 (3-1-1)	320d±16	368c±76	350c±12	351c±25	342c±54
5 (2-0-3)	433c±31	521b±32	530b±75	390b±82	339c±15
6 (2-2-1)	445c±43	512b±25	376c±62	394b±88	357c±22
7 (1-1-3)	445c±43	426b±51	450c±50	597a±207	511a±7
8 (1-2-2)	463c±24	485b±50	431c±25	411b±65	323c±198
9 (0-4-1)	701a±31	624a±6	623a±6	410b±38	449b±12

Means with the same letter in the same column are not different according to Tukey's test ($p < 0.05$).

Available potassium was affected by the different proportions of the 3 types of organic matter at the beginning of the experiment (time zero), specifically with proportions (2:3:0) and (0:4:1) likely due to the addition of earthworm compost. After 10 days of incubation, available potassium increased in several treatments with similar results. In general, the available potassium increased during the incubation time; however, the available potassium decreased after 40 days of the maturation phase.

Increases in available potassium in soil and in organic substrates may occur in mixed biofertilizers produced with phosphate, potassic rocks and organic matter (earthworm compost) and may have residual effects as reported by Stamford et al. (2014). The effects of the mixed biofertilizer may be caused by sulfuric acid release by the sulfur oxidative bacteria *Acidithiobacillus*, which increases the availability of phosphorus and potassium contained in the natural rocks used to produce the mixed biofertilizers.

Santos, Tiritan, Foloni, and Fabris (2010) reported that sugarcane mud cake is an organic compound that is also rich in potassium but that varies in its composition according to the variety of sugarcane and plant maturation.

Soluble sodium

Significant differences in soluble sodium were observed in the treatments with different proportions of the 3 types of organic matter (Table 6).

Table 6. Soluble sodium in the biofertilizers with different proportions of organic matter (sugarcane mud cake: earthworm compost: crop residue), inoculated with the diazotrophic bacteria *Beijerinckia indica* (strain NFB 10001), after incubation in the maturation phase with various times (0. 10, 20, 30, and 40 days) at room temperature (28 ± 2°C).

Treatment	Incubation time (days)				
(Proportions)	0	10	20	30	40
	Exchangeable sodium (mg dm^{-3})				
1 (5-0-0)	84c ±10	196b±32	102b±9	104d±19	93d±4
2 (2-3-0)	513b±167	366a±12	398a±58	224c±6	178c±58
3 (3-2-0)	494b±133	265a±77	338a±91	485a±259	379a±32
4 (3-1-1)	187c±71	219a±25	292a±25	160d±19	272b±101
5 (2-0-3)	792a±45	347a±2	309a±132	471a±175	255b±19
6 (2-2-1)	260c±84	334a±32	275a±102	228c±64	250b±25
7 (1-1-3)	753a±130	256a±25	283a±25	352b±175	139d±48
8 (1-2-2)	233c±38	288a±45	352a±65	201c±51	294b±64
9 (0-4-1)	270c±16	316a±6	339a±103	361b±218	234b±38

Means with the same letter in the same column are not different according to Tukey's test ($p < 0.05$).

At the initial time (time zero), higher levels of soluble sodium were observed in treatments (2:0:3) and (1:1:3); those treatments contained greater amounts of the organic matter crop culture. The other treatments contributed less soluble sodium to the substrate. The increase in soluble sodium during the period from 10 to 20 days was similar and did not significantly differ. This finding is not of great significance because the increase in soluble sodium in soil and in organic substrates is problematic; excess Na^+ can increase the osmotic potential of the soil and reduce the availability of water, which reduces water infiltration into the soil and can have negative consequences on seed germination and plant growth.

Total N and C

The total N and C did not significantly differ ($p > 0.05$) between the incubation periods in the maturation phase, and the different treatments only had individual effects of the organic matter proportions on these nutrients (Figures 1 and 2).

The total N increased an average of 8.58 to 11.61 g kg^{-1} in the treatments and proportions 2:0:3, 1:2:2, and 0:4:1 were significantly different (Figure 1) and superior to the other treatments. These results differed from those of Stamford et al. (2014), who observed significant differences, in total N in soil when various fertilizer treatments (rock biofertilizers, mixed organic biofertilizers, and soluble fertilizer) were applied compared to earthworm compost (20 t ha^{-1}).

All applied treatments increased total C, showing a maximum increase in total C of 80 to 112 g kg^{-1} (Figure 2). The use of different proportions of the 3 types of organic matter to produce the biofertilizers may have promoted an increase in nutrients, particularly N and C because these substrates normally have a higher quantity of these elements that are released by the decomposition and mineralization of organic matter.

Increased total C in soil was reported by Stamford et al. (2011) using different fertilizer treatments (mixed biofertilizer, rock biofertilizers and soluble fertilizer) in grapevine (*Vitis vinifera*) grown in the San Francisco Valley, Brazil (Brazilian semiarid region).

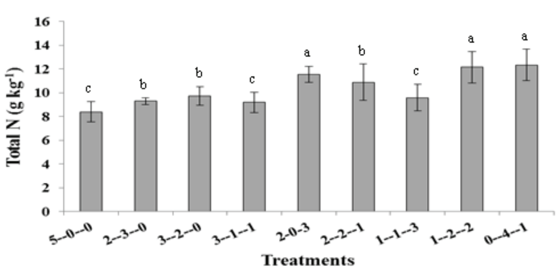

Figure 1. Total N in the biofertilizers with different proportions of the 3 types of organic matter (sugarcane mud cake: earthworm compost: crop residue), inoculated with selected diazotrophic bacteria *Beijerinckia indica* (strain NFB 10001), during the maturation phase under various incubation times (0, 10, 20, 30, and 40 days) at room temperature (28 ± 2°C).

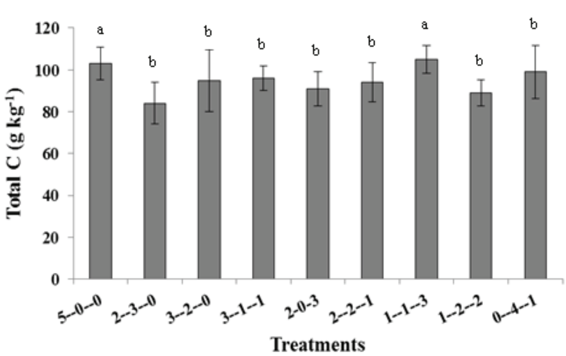

Figure 2. Total carbon in the biofertilizers with different proportions of the 3 types of organic matter (sugarcane mud cake: earthworm compost: crop residue), inoculated with the selected diazotrophic bacteria *Beijerinckia indica* (strain NFB 10001), after incubation for various times (0, 10, 20, 30, and 40 days) at room temperature (28 ± 2°C).

Conclusion

The different proportions of the organic matter used to produce organic biofertilizer contributed to increase nutrients availability in the substrates (biofertilizers), especially with proportions (5:0:0) and (3:2:0) for Ca, Mg and P; proportions (2:3:0) and (2:0:3) for K and N, respectively. The study confirmed the estimative of nutrient availability after residue addition which increased with the incubation period of 30 days. Biofertilizers with optimal organic matter proportions may be used as alternatives to soluble NPK fertilizers.

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